

# The Role of Calcium in Calprotectin Dimerization as a Cancer Biomarker

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**Background:** S100A8 and S100A9 as two subunits of heterodimeric calprotectin are identified mainly in leukocytes and are involved in inflammatory processes and several cancerous pathogens. This study was performed in order to evaluate the interaction of recombinant calprotectin subunits and to estimate calprotectin's tertiary and secondary structures.

**Objectives:** The aim of this study was to investigate the effects of calcium in calprotectin dimerization as a cancer biomarker.

**Methods and Materials:** Heterodimeric calprotectin was formed with incubation of recombinant S100A8 and S100A9 subunits in the presence of Ca (1 mM), at 25 °C for 15 minutes. Tertiary and secondary structures of S100A8, S100A9 and their complex were investigated, using fluorescence and circular dichroism (CD) spectroscopy, respectively.

**Results:** Interaction of S100A8 and S100A9 in the presence of Ca<sup>2+</sup> were revealed by decreasing the emission intensity of intrinsic fluorescence and increasing of the external fluorescence and also changes in the CD spectra of subunits after Ca<sup>2+</sup> interactions.

**Conclusions:** The expression of recombinant calprotectin, as an effective protein, can help in diagnosis or treatment of inflammatory and cancer processes in the future. Furthermore, Ca<sup>2+</sup> induced a partial change in secondary and tertiary structure of calprotectin subunits and this change is probably necessary for protein dimerization.

**Keywords:** Calcium; Calprotectin; Biomarkers; Cancer; Fluorescence Spectroscopy

## 1. Background

Calprotectin is a member of the S100 family of proteins, and is a marker of inflammation and a calcium and zinc-binding protein. Expression of calprotectin has been reported mainly in neutrophils (30-60% in the cytosol), followed by monocytes and macrophages (mainly associated with membranes), and to a lesser extent in other cells. Expression of S100A8 and S100A9 and hence calprotectin are induced following recruitment of macrophages to inflammatory sites; calprotectin is not stored in tissue macrophages (1). The calprotectin structure is comprised of a hetero-dimer with two calcium-binding chains and two calcium-binding sites per chain. The heavy chain is a 14 KD protein, also known as MRP14/S100A9/P14/L1H and the light chain is an 8 KD protein, also known as MRP8/S100A8/L1L/P8 (1-3). The chains bind non-covalently in the presence of calcium. Other compounds namely, hetero- or homo-dimer of the two chains, tetrameric or more monomers per polymer chain have also been identified. Twenty-one S100 genes, including those for calprotectin, are clustered on human chromosome 1q21. Until now homo-dimer of S100 proteins including S100A8 and S100A9 have been reported; the primer functional form was reported to be heterodimeric con-

sisting of antiparallel arrangement of S100A8/S100A9 known as calprotectin, which is induced in the presence of calcium. S100A8 and S100A9 are produced primarily in myeloid cells and cells triggered by inflammation of myeloid lineage with the exception of lymphocytes. S100A9 gene deletion leads to the loss of S100A8. Expression of S100A9 and S100A8 proteins in phagocytes are associated with a set of actions in the innate immune system. The expression of these proteins occur during differentiation of macrophages and dendritic cells; both proteins can be simultaneously expressed in monocytes, endothelial cells, keratinocytes and epithelial cells by several mediators such as interleukin (IL1)-alpha, IL1-beta, IL10, IL22, tumor necrosis factor (TNF) alpha and lipoteichoic acid (LPS) (4). Different roles have been reported for calprotectin, including; anti-microbial, cytotoxicity, cytokine-like activity, anti-proliferation, induction of apoptosis, chemotactic effects, leukocyte-endothelium interaction, cell adhesion, immune regulation, inflammation and coagulation responses. Levels of calprotectin were found increase following infections and inflammatory disease states (1, 3). Normally, calprotectin has been reported to be at a concentration of about 5 mg in plasma and 2